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OBSERVATIONS ON PURE CULTURES OF SOME ASCOMYCETES AND BASIDIOMYCETES.

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Most fungi with the exception of those which are obligate parasites like Rusts and Mildews may be grown upon artificial media of one kind or another, choice being usually made of the media most nearly approaching in character those on which the fungi grow in nature. Brefeld, the magician among mycologists who has induced so many of the higher fungi to unfold their secrets, investigated for many years with extraordinary success the life-histories of fungi by the method of pure culture, and those who follow in the path which he has so clearly opened up will always have to refer to his series of brilliant monographs. In his researches, Brefeld used many different kinds of media including decoctions of dung, fruits, potatoes, and meat, among liquid media; and dung, sawdust, bread, as well as gelatine and agar-agar containing various decoctions or mineral substances, among solid media.

Brefeld was not successful in inducing the formation of fruit bodies in the case of some of the higher fungi he cultivated and more recent investigators have obtained greater success in this direction, especially with fungi which live upon woody tissues. Costantin and Matruchot* were the first to grow in pure culture upon blocks of wood one of the higher Basidiomycetes up to the stage of the formation of sporophores. The fungus they worked with was *Collybia velutipes*, and sporophores of normal form though small in size were produced in their cultures on wood of *Robinia Pseudacacia*. Immediately after their success Marshall Ward† cultivated *Stereum hirsutum* in the same manner upon sterilised blocks of Horse-Chestnut wood. The spores were allowed to germinate in agar plates and the young mycelia were transferred to blocks of wood. Since then many other wood-destroying fungi have been cultivated in pure culture to the stage of the formation of fruit bodies. Thus Biffen‡ has again ob-

* Costantin et Matruchot, "Culture d'un Champignon lignicole." Comptes Rendus, Vol. 119, p. 752.

† Ward, H. M. "On the Biology of *Stereum hirsutum*." Phil. Trans. Roy. Soc. 1897.

‡ Biffen, R. H. "On the Biology of *Collybia velutipes*," Journ. Linn. Soc. xxxiv.

tained the formation of sporophores of *Collybia velutipes* in pure culture, and both Miss Wakefield* and I† those of *Stereum purpureum*, Miss Bayliss‡ those of *Polystictus versicolor*, Learn§ in America those of *Pleurotus ostreatus and Pleurotus ulmarius.*

In growing many of these forms upon wood considerable attention has been paid to the manner in which the lignified tissues are attacked by the fungus in pure culture, and in growing *Stereum hirsutum* in this way it was Marshall Ward's primary object to investigate the process of delignification in a detailed manner. It is obvious that such processes can be investigated more critically by this means than by the examination of tissues of trees attacked by fungi in nature. Considerable light has been thrown upon the manner in which fungi destroy woody tissues by the prosecution of this method. Biffen has shown that the mode in which the common fungus *Collybia velutipes* attacks wood is essentially different from that in which *Stereum hirsutum* attacks it. The latter fungus causes delignification of the walls at the beginning of attack, while *Collybia velutipes* normally consumes the cellulose and leaves the lignin behind.

Many other facts of biological interest have been discovered by the cultivation of fungi under artificial conditions. Spore stages which are rarely or never seen in nature have been found to occur in the life-histories of many fungi when grown in culture media. Thus a large number of the higher fungi investigated by Brefeld‖ produce conidia of various kinds in the early stages of development, e.g., *Peziza vesiculosa* forms conidiophores when grown in culture media and Miss Marryat¶ has shewn that *Pleurotus subpalmatus* when grown upon Elm blocks gives rise to chlamydospores in the vessels of the wood.

It is frequently difficult to obtain the highest spore stage of a fungus when it is grown upon an artificial medium, especially in the case of the higher fungi. Much can sometimes be accomplished in this respect by changing the character or concentration of the medium. Some fungi which are sterile when grown on gelatine media fructify if the substratum consists of agar-agar, some which produce only vegetative mycelium when grown in a highly nutritive medium fructify readily if partly starved. Never-

* Wakefield, E. M. "Die Bedingungen der Fruchtkörperbildung bei Hymenomyceten." Naturw. Zeitsch. f. Forst. und Landwirt. 1909.

† Brooks, F. T. "Silver-leaf Disease." Journal Agric. Science, 1911.

‡ Bayliss, J. S. "On the Biology of *Polystictus versicolor.*" Journ. Econ. Biol. 1908.

§ Learn, C. D. "Studies on *Pleurotus ostreatus* and *Pleurotus ulmarius.*" Ann. Myc. 1912.

‖ Brefeld, O. "Untersuchungen aus der Gesammtgebiete der Mykologie," Heft X. pp. 333-337.

¶ Marryat, D. "Chlamydospore-Formation in the Basidiomycete, *Pleurotus subpalmatus,*" *New Phyt.* 1908.

theless until the present time comparatively few Ascomycetes and Basidiomycetes have been cultivated up to the stage of the formation of asci or basidia. Little is yet known as to the conditions which favour the formation of the ascocarps and sporophores of the higher fungi. It is obvious that before fructifications can arise, the vegetative mycelium must have developed sufficient strength to feed them, and it is likely that the failure to obtain formation of sporophores in the case of some Basidiomycetes is primarily due to the fact that the cultures have not been sufficiently large to enable the mycelium to develop the requisite vigour. When sporophores are produced in culture tubes or flasks they frequently lack the form and consistency of fructifications which develop in nature. This may be attributed to the different conditions under which growth takes place in the two cases. In the culture tube the fructifications develop in a still atmosphere which is often saturated with water vapour, whereas fructifications which arise out of doors are subjected to air currents which tend to increase transpiration. Miss Bayliss* has shewn that when *Polystictus versicolor* is grown in culture tubes, only rudimentary sporophores develop, but if, after the mycelium is well established, the tubes are exposed to atmospheric conditions sporophores of normal shape are formed. As the cultures become older, increasing desiccation may be a factor in the development of fruit bodies for it is well known that certain fungi produce under moist conditions only sterile mycelium. Thus *Merulius lacrymans* when growing in an excessively humid atmosphere vegetates in a most luxuriant manner but does not form fructifications. Light is another factor which exercises a great influence on the mode of development of fructifications in certain of the higher fungi, though in some e.g., the Mushroom, fruit bodies develop quite well in complete darkness. Buller† has shewn that the stimulus of light is necessary for the development of the pileus in *Polyporus squamosus* and *Lentinus lepideus* when growing on woody tissues naturally infected by the mycelia of these forms. The same phenomenon can be observed in some of the Basidiomycetes which have been cultivated under critical conditions. Thus in cultures of *Coprinus plicatilis*, Brefeld demonstrated that in the absence of light the rudimentary fructifications do not form a pileus, and in *Coprinus nycthemerus* no trace of sporophores develops in darkness. In *Stereum hirsutum* on the other hand Marshall Ward‡ found that no difference was observable in regard to the formation of rudimentary sporophores between cultures kept in light and those in darkness and the same is true of my cultures of *Stereum pur-*

* Bayliss, Jessie S., loc. cit. p. 20.
† Buller, A. H. R. "Researches on Fungi," 1909.
‡ Ward, H. M., loc. cit.

pureum. We know little of the influence that variations in temperature have on the development of the higher fungi though it is probably well marked in them as in the lower fungi and imperfect forms. In most methods of growing pure cultures the conditions of aeration are poor, though it is not easy to say what influence this exerts on development, especially as it is difficult to separate the influence of desiccation from that of good aeration. Lakon* found that in pure cultures of certain Coprini the formation of sporophores did not take place as long as the atmosphere remained saturated and stagnant, but when a stream of air was passed through the cultures, sporophores developed readily. Brefeld certainly preferred to use substances such as bread and sawdust rather than more compact materials like wood, gelatine, etc., on account of their better aeration.

In several of the higher fungi it has recently been shewn that different strains of one and the same species exist which behave differently when grown under the same cultural conditions, especially as regards their capacity to form the ascal or basidial stage. Miss Wakefield† in her work on *Schizophyllum commune* and *Stereum purpureum* found that forms isolated from one substratum readily gave rise to sporophores in pure culture, whereas others obtained from different situations and treated in the same way failed to develop fruit bodies. Shear‡ in America has proved that the same phenomenon holds good for the Ascomycete, *Glomerella cingulata.* This fungus causes a disease of the leaves and fruits of many plants and forms both conidia and perithecia. A certain number only of isolations from fresh material resulted in the development of perithecia in artificial culture, the others invariably giving only the conidial stage even when conditions of culture were changed in order to try to induce the formation of asci. Thus in this fungus we may speak of one strain which produces in culture the conidial stage only and of another strain which forms both the conidial and perithecial stages. In such cases as the above no morphological differences can be observed in the fungi at the time of being obtained from different sources. Quite recently Harper and Field§ in their work on *Diaporthe Batatatis* which causes a rot of sweet potatoes in the United States have found the existence of the same phenomenon, i.e., one strain or variety of the fungus which readily

* Lakon, G. B. "Die Bedingungen der Fruchtkörperbildung bei *Coprinus*." Ann. Myc. V. 1907.

† Wakefield, E. M. loc. cit.

‡ Shear, C. L. and Wood, Anna K., "Studies of Fungus Parasites belonging to the genus *Glomerella*." U.S. Dept. Agric. Bur. of Plant Industry, Bull, 252.

§ Harper, L. L. and Field, E. C., "A dry rot of Sweet Potatoes caused by *Diaporthe Batatatis*." U.S. Dept. Agric. Bur. of Plant Industry, Bull, 281.

produces perithecia in culture and another giving rise only to pycnidia.

The cultures of fungi which I am about to describe have all been grown upon wood, as this is the substratum upon which these particular forms are found in nature. The mode of establishing the cultures will be briefly described. In the first place small blocks of wood of the kind usually attacked by the fungus in nature are placed in culture tubes, each piece of wood being allowed to rest upon a thick plug of cotton wool saturated with water. It is found preferable to use tubes which have a water reservoir at the bottom so that the cultures do not become dry too rapidly. The ends of the tubes are plugged with cotton wool and then the group of tubes is sterilised in a boiler or autoclave on two or three successive days. It is advisable to avoid the use of very high temperatures in the process of sterilisation as such may have a deleterious influence upon the substances in the wood which subsequently provide food for the fungus. After the preparation and sterilisation of the tubes, care must be taken to obtain in a state of purity the spores of the fungus which it is desired to cultivate. This can generally be done by taking advantage of the method of spore discharge for the particular fungus in question. Thus by suspending the hymenial surfaces of Basidiomycetes over sterilised glass slides under suitable conditions for the formation of spores one usually obtains a good spore deposit; in a similar manner the fructifications of many Ascomycetes may be allowed to project their spores on to sterilised cover slips suspended above the ascocarps. Having obtained the spores in a state of purity they are transferred directly to blocks of wood in culture tubes by means of a sterilised needle or scalpel, care being taken to prevent contamination of the tubes by other organisms at the time of withdrawing the plugs of cotton wool. The inoculated tubes are then placed in a dull light at ordinary temperature and are observed at intervals. Some weeks occasionally pass before the production of an evident mycelium, and more than once tubes have been thrown away because it seemed likely that the fungus was not going to develop although it has subsequently transpired that sufficient time had not been allowed for development to take place. If this method is followed with care, contaminations are of rare occurrence, and in the majority of the fungi I have endeavoured to cultivate in this way there has been no difficulty in establishing good mycelial growths.

The first fungus of which pure cultures will be described is:—

Chlorosplenium aeruginosum (Oed.) de Not.

which is well known as the cause of the greening of various kinds of wood such as Oak, Beech, Ash, Hazel, Alder, and Birch. This

wood is applied to various ornamental uses by turners at Tunbridge Wells and elsewhere, and articles made of it are known as Tunbridge Ware. Fructifications of this fungus were obtained during the foray of this Society at Forres in 1912, and Mr. H. H. Thomas kindly gave me a few ascocarps on returning to Cambridge. These were rather shrivelled at the time but rapidly recovered on being moistened, and, the spores being violently ejected from the asci, it was easy to obtain them in a pure state by suspending sterilised coverslips over the cups. The spores were transferred to blocks of Ash wood upon which a white mycelium began to develop in a few days. Soon after, the mycelium turned a beautiful green colour similar to that of the fructifications, and gradually spread over the wood and into the cotton wool at the bottom of the tube. The mycelium has since been transferred to other tubes in which it is growing vigorously. When the first cultures were established on blocks of Ash wood, spores of the fungus were also placed on Plum wood. On this material only a feeble growth has resulted, and the mycelium after an interval of some months lacks the brilliant colour which is associated with active growth on Ash wood. I do not know of any record of the fungus being found on Plum wood in nature, and this substratum is evidently unsuitable for its development. Green mycelium taken from a culture on Ash wood has been transferred to blocks of Plum wood but it has invariably lost its colour in the course of development on the latter. Green mycelium from a pure culture has been transferred to blocks of Oak wood upon which it has grown well. The same mycelium placed upon Pine wood develops with exceeding slowness but it remains green in colour.

It will be remembered that a controversy raged for many years as to whether greening of wood was due to this fungus or whether the wood could become green apart from its action. The evidence was strongly in favour of the fungus being the sole cause of the greening, but when de Bary wrote his Comparative Morphology and Biology of the Fungi in 1884 the matter was still open to discussion. In 1891 Brefeld,* writing on cultures of mycelium obtained in nutritive solutions from spores, pointed out that of two mycelial growths which he established one was colourless and the other bright green, from which he concluded that the greening of wood was caused by this fungus.

If any doubt still exists as to *Chlorosplenium aeruginosum* being the cause of the greening of wood it can be set at rest by my cultures, for the blocks of Ash wood and Oak wood which have been covered by the mycelium for some months are green

* Brefeld, O. "Untersuchungen aus der Gesammtgebiete der Mykologie." Heft. X.

throughout. Sections shew that the green hyphae of the fungus have penetrated to the centre of the block and are especially abundant in the vessels. The walls of some of the elements of the wood assume a greenish colour but the chief factor in the colouring of the wood is the accumulation of lumps of a green amorphous substance in the different elements especially in the cells of the medullary rays. This substance is probably a bye product caused by the action of the fungus on the tissues. The action of the fungus in pure culture therefore agrees closely with its action on naturally infected wood as described by Prillieux in 1877.† Very little delignification has yet resulted in my pure cultures because the fungus has only acted on the wood for a limited period, but Prillieux has shewn that in nature, when the action of the fungus is prolonged, delignification is effected, and by this the wood loses its value for the purpose of ornamental work. It is well known that the colour of this fungus is stable, and resists the action of many chemical agents. It is insoluble in alcohol and ether although it is acted upon by chloroform. Mineral acids have scarcely any effect upon it but the colour is taken away by alkalis. In my cultures conidia similar to those described by Brefeld have been produced, but no formation of spermogonia as described by Tulasne‡ or of ascocarps has yet taken place.

Daldinia concentrica (Bolt.) Ces. et de Not.

The large black fructifications of this fungus are of frequent occurrence on stumps and branches of Ash trees in this country. At maturity the walls of the asci break down and the spores are extruded in threads from the orifices of the perithecia which stud the surface of the fruit-bodies. Spore discharge continues for weeks and the quantity of spores produced is enormous, large black masses of them accumulating round the fructifications when kept in the laboratory. The mycelium of the fungus grows well on blocks of Ash wood on which spores have been placed. At first white in colour the mycelium becomes tawny with age, and where it comes in contact with the glass tube it aggregates and becomes blackish in colour similar to the hyphae which form the matrix of the stroma. The mycelium in pure culture produces branched conidiophores from which elliptical, hyaline spores, 6-8 × 3-4μ in dimensions, are abstricted; these spores are sometimes grouped together in small heads. Möller,* who investigated in Brazil the biology of this fungus, obtained a similar

† Prillieux, "Sur la coloration en vert du bois mort," Bull de Soc. bot. de France, 1877.

‡ Tulasne, Carpologia, III. Paris, 1865.

* Möller, A. "Phycomyceten und Ascomyceten." In Schimper A.F.W. Bot. Mitth. aus den Tropen Heft 9. Jena, 1901.

conidial stage by sowing the ascospores in nutritive solutions, and in the closely allied *Hypoxylon fuscum*, which Brefeld grew from ascospores, similar conidiophores developed under cultural conditions. A conidial stage of *Daldinia concentrica* which occurs in nature is described in Rabenhorst's Kryptogamen-Flora, and Dr. Bayliss Elliott informed the Society at its meeting at Haslemere that she had recently had this stage under observation. In these cultures the hyphae of *Daldinia concentrica* readily penetrate the wood, being especially abundant in the vessels.

Hydnum coralloides (Scopoli) Fr.

In September 1912 some very fine fructifications of this fungus developed on dead portions of an Ash tree in a private garden in Cambridge. The Ash log on which the fungus grew was only slightly decayed. One of the fructifications was 9 inches in diameter and 4 inches in height and looked exactly like a mass of white coral. Fructifications of *Hydnum coralloides* persist longer than one would be led to believe from their delicate appearance; their form is retained for more than a fortnight and spore discharge continues at least ten days. A fructification was suspended over a clean sheet of glass and the following morning spores deposited on the glass were transferred to tubes containing Ash wood. A thin white mycelium became evident in three weeks and towards the end of December two branched fructifications with well developed spines were formed on the wood in one of the tubes. These fructifications though small, being only an inch high and half an inch broad, were closely similar in structure to those occurring in nature. The following February another culture produced a sporophore but this was less normal in appearance than those already described. Other tubes have since developed fructifications of rather an abnormal type. Subsequent transfers of the mycelium to other blocks of Ash wood resulted in the formation of sporophores which were more abnormal in form than those first produced and upon which spines were developed in a rudimentary manner.

During October 1913 another large fruit body of this fungus developed on an Ash log in the same garden.

Fomes igniarius (Linn.) Fr. var. *pomaceus* (Pers.) Fr.

This fungus is commonly met with in this country on the trunks and branches of Plum trees, Damsons, and Greengages, especially the two latter. It frequently causes the death of limbs of these trees but as its progress is slow it can easily be checked in fruit plantations by cutting away affected parts.

Plum wood affected by the fungus in nature becomes dark brown in colour during the early stages of attack on account of

the accumulation of gum-like substances in the cavities of the cells. For a long time I was unable to obtain a spore deposit from the fruit bodies, but success ultimately was met with and pure cultures were established upon plum wood. The mycelium of this fungus is white in the early stages of development, but as time proceeds dense yellowish brown aggregations appear in certain parts of the tubes and these are doubtless rudimentary sporophores, although spore-bearing surfaces have not yet developed. The mycelium permeates the wood and causes the accumulation therein of gum-like substances as in the case of naturally infected branches. Hartig* has described the action of *Fomes (Polyporus) igniarius* on naturally-infected Oak wood, and though there has yet been little delignification in my cultures the action of the fungus on the elements of the wood appears to be closely similar in kind to that described by him. No conidial stage has been observed in the pure cultures.

Pleurotus ostreatus (Jacq.) Fr.

The oyster shell fungus, as *Pleurotus ostreatus* is popularly called, is frequently seen growing from the trunks and branches of Elm trees in this country, and it is commonly found also in North America where Learn† has recently described its action upon naturally-infected wood of Maple *(Acer saccharum)*. This fungus has been grown in the usual manner on blocks of Elm wood. Its mycelium is white in colour, and in my culture is characterised by an extremely profuse growth. So luxuriant has this growth been that the lower halves of the culture tubes have become completely filled with mycelium.

Fructifications began to appear in each of the culture tubes after about four months and have continued to develop successively. Sometimes the fruit bodies arise in groups, sometimes they are single. The pilei are smaller than those produced in nature and the stalks are relatively longer. Gills are well developed and appear to be normal in structure; they are decurrent as in the fruit bodies which develop in nature. Learn speaks of the production of abnormal sporophores in his cultures of this fungus on bass-wood and oak but he does not say if gill surfaces were formed on them.

Hyphae penetrate the blocks of wood and delignify it in the manner described by Learn for the naturally infected wood.

Coprinus sp.

Two years ago a charred log of wood partially covered with a mycelium belonging to an *Ozonium* was sent to me. The log

* Hartig, R. "Zersetzungserscheinungen des Holzes." Berlin, 1878.
† Learn, C. D. loc. cit.

was placed on the roof of the Botany School, Cambridge, to see whether in the course of time any fructifications would develop in connection with the *Ozonium*. No change occurred for more than a year, although the latter remained constantly present, but during June 1913 fructifications of a *Coprinus* began to develop from the *Ozonium* mycelium. It was easy to demonstrate the connection between the tawny mycelium of *Ozonium* and the young sporophores of the *Coprinus*. The pileus was $\frac{3}{4}$-1 inch in diameter and about the same in height; it was yellowish in colour and covered with small scales when young. The gills were provided with large, colourless cystidia which resembled those of *Coprinus atramentarius* described by Buller.* The stipe was 2-3 inches long and $\frac{1}{8}$ inch in diameter and from the base of it conspicuous yellow strands radiated outwards in all directions. Mr. Carleton Rea tells me the species was probably *Coprinus radians*. Fructifications appeared again on the same log during Novemer 1913. A deposit of spores was obtained in the usual manner and some were transferred to blocks of Elm wood. A white mycelium soon appeared and during the last few weeks the tawny *Ozonium* has begun to develop so that the *Ozonium* has been reconstructed from the spores of *Coprinus* grown in pure culture. This is the first time this has been accomplished as far as I am aware, although the reverse process of tracing the *Ozonium* mycelium into fructifications of a *Coprinus* has been described before. Thus Plowright† stated in the Transactions of this Society published in 1902 that he had traced the connection between *Ozonium auricomum* Link and fructifications of *Coprinus domesticus*.

I hope to continue investigations on the cultures which have been briefly described in this paper.

* Buller, A. H. R. "The cystidia of *Coprinus atramentarius*." Ann. Bot. 1910.

† Plowright, C. D. "*Ozonium auricomum*, Link." Trans. Brit. Myc. Soc. 1900-1.